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EVALUATION OF THE EFFECTS OF THE HYDRO-ETHANOLIC ROOT EXTRACT OF *ZANTHOXYLUM ZANTHOXYLOIDES* ON HEMATOLOGICAL PARAMETERS AND OXIDATIVE STRESS IN CYCLOPHOSPHAMIDE TREATED RATS

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Abstract

Background: The use of cyclophosphamide in cancer therapy is usually associated with challenging immunosuppression which exposes patients to increased risk of anemia and necessitating preventive measures during therapy. This study was carried out to investigate the efficacy of the hydro-ethanolic extract of the root of *Z. zanthoxyloides* in preventing and/or improving cyclophosphamide induced myelosuppression and oxidative stress in rats.

Materials and Methods: Animals were divided into 6 groups of 6 rats each and were pretreated oral doses of 75, 150 and 225 mg/kg of the extract for 7 days and then co-administered with 2.5 mg/kg cyclophosphamide for 28 days.

Results: The LD₅₀ of the extract was found to be 1682.3 mg/kg. Phytochemical analysis of the plant extract showed the presence of tannins, saponins, alkaloids and flavonoids, glycosides, terpenoids and phenols. In the anti-oxidant enzyme assay, CAT was significantly ($p < 0.05$) increased for animals treated with 150 mg/kg+CP compared to 75 mg/kg+CP and 225 mg/kg+CP. GPx was significantly ($p < 0.01$) increased in rats treated with 75 mg/kg+CP compared to 150 mg/kg+CP and control. SOD was significantly ($p < 0.01$) increased in rats treated with 75 mg/kg+CP compared to the control. WBC was significantly ($p < 0.05$) reduced for 225 mg/kg, 225 mg/kg+CP ($p < 0.001$), 150 mg/kg+CP ($p < 0.001$), 75 mg/kg+CP ($p < 0.001$) and CP administered rats ($p < 0.001$) respectively compared to the control. LDL and CHOL were significantly reduced ($p < 0.05$) for rats treated with 75 mg/kg+CP, 225 mg/kg+CP and 225 mg/kg.

Conclusion: Findings from this study demonstrates that the hydro-ethanolic root extract of *Z. zanthoxyloides* could be beneficial in hyperlipidemia and in cases of malignancies with abnormal cholesterol metabolism an effect which may be mediated via combating oxidative stress.

Key words: cyclophosphamide, phytochemical, antioxidant, hematological, hyperlipidemia

List of Abbreviations: EDTA: Ethylenediamine-tetra acetate; MDA: Malondialdehyde; PCV: Packed cell volume; RBC: Red blood cell; HGB: Hemoglobin; WBC: White blood cell; ALT: Alanine transaminase; AST: Aspartate transaminase; CHOL: Cholesterol; LDL: Low density lipoprotein; HDL: High density lipoprotein; GSH: Reduced glutathione; SOD: Superoxide dismutase; CAT: Catalase; CP: Cyclophosphamide

Introduction

Many developing countries of the world are endowed with vast resources of natural products. This priceless heritage which includes medicinal plants have been used by people for centuries as drug substances for relief from illnesses, as health-care products, fragrances, flavors, sweeteners and as materials for pest control. And today, plants appear to be the almost exclusive source of drugs for the majority of the world's population, with substances derived from higher plants constituting about a quarter of all prescribed medicines (Adesina, 2005).

Zanthoxylum zanthoxyloides is a component of the rainforest vegetation of Southern Nigeria, and is represented by eleven species. A few of these species occur more abundantly in the savannah and dry forest vegetation of South-western Nigeria. Throughout West Africa, the root, stem bark and leaves of *Z. zanthoxyloides* are commonly used in traditional medicine. They are considered antiseptic, analgesic and diaphoretic. Root or stem bark macerations, decoctions or infusions are widely taken to treat malaria, fever, sickle cell anemia, tuberculosis, paralysis, edema and general body weakness. Studies have also shown it to possess high antioxidant property (Andersson et al., 1996; Sofowora et al., 1975). Traditional healers throughout Nigeria have used species of the *Zanthoxylum* for the treatment of a wide range of disorders, including toothache, urinary and venereal diseases, rheumatism and lumbago. The plant is known for its anti-oxidative, anti-inflammatory, antisickling, antibacterial, antiviral, anti-hepatotoxicity, anti-allergic, anti-tumoral and antihypertensive properties (Adesina, 2005; Andersson et al., 1996; Sofowora et al., 1975). Hence, this study was carried out to determine the acute toxicity of the hydro-ethanolic root extract of *Z. zanthoxyloides* as well as its effect on serum biochemical markers, hematological and antioxidant indices in cyclophosphamide treated rats.

Materials and Methods

Plant Materials

The fresh samples of roots of *Z. zanthoxyloides* used in the study were obtained commercially from Mushin market, Lagos

state, Nigeria. The plant was identified and authenticated in the Herbarium unit of the Department of Botany, University of Lagos, Akoka, Lagos state.

Laboratory Animals

Laboratory rats weighing between 120-130 g and mice weighing between 25-30 g of the male and female sex used in this study were obtained from the Laboratory Animal Centre, College of Medicine, University of Lagos, Lagos, Nigeria. The animals were kept under standard environmental conditions (23–25°C, 12 h/12 h light/dark cycle) and fed with standard rodent pellet (Livestock Feed PLC, Lagos, Nigeria) and water *ad libitum*. The experimental protocol adopted in this study was approved by the Experimentation Ethics Committee on Animal Use of the College of Medicine, University of Lagos, Lagos, Nigeria and was in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (NIH, 1996).

Extraction

The roots were washed with clean water and air-dried for 7 days until constant weight was obtained. The dry root-bark was prepared by chopping the bark into small pieces and grinded with a laboratory mill into coarse powder. The powder was soaked with 10 L of 96% ethanol and 4.29 L of distilled water for 72 h and then extracted. The filtrate was then concentrated by drying in an oven at 40 °C for 72 h.

Acute Toxicity

The acute toxicity study was carried out using 25 male albino mice. They were fasted for 12 h and administered the extract at doses of 1 - 5 g/kg orally.

Preliminary Phytochemistry Analysis

The hydroethanolic extract of *Z. zanthoxyloides* was screened for the following phytochemical principles; alkaloids, saponins, cardiac glycosides, anthraquinones, tannins and phlobatannins using simple established methods as outlined by Odebiyi and Sofowora (1978).

Test Samples

Weighed quantities of extracts were suspended in 3% v/v Tween 20 to prepare a suitable dosage form.

Drugs

Cycloxan® (Biochem–pharmaceutical industries Ltd., Mumbai) containing 500 mg cyclophosphamide was procured from the market and dilutions were made using sterile water for injection as mentioned on the label of the marketed product.

Grouping

Animals were randomly divided into six groups of six animals each, five experimental groups and one control group. Group I served as the control and received 10mL/kg of 3% v/v Tween 20 for 28 days. Group II (Cyclophosphamide group) received 2.5 mg/kg of cyclophosphamide for 21 days. Groups III, IV and V were administered hydro-ethanolic extracts of the root of the plant at the doses of 75 mg/kg, 150 mg/kg and 225 mg/kg (per oral) daily for 7 days followed by co-administration with cyclophosphamide for 21 days. Group VI was administered only 225 mg/kg of the extract for 28 days.

After termination of oral treatments, blood samples were collected by retro orbital puncture for hematological and anti-oxidants study.

Hematological Assessment

Blood samples from experimental animals were collected into EDTA (ethylenediamine-tetraacetate) bottles and analysed using standard procedures. Erythrocyte (RBC), hemoglobin (Hb), packed cell volume (PCV), platelet count, and total and differential leukocyte (WBC), were determined using automated hematology analyzer.

Measurement of *In Vivo* Antioxidants and MDA Levels

The following antioxidant enzyme activities were determined using the spectrometer as follows:

Determination of Superoxide Dismutase

Superoxide Dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the

increase in absorbance at 480nm as described by Sun and Zigma (1978). The reaction mixture (3ml) contained 2.95ml 0.05M sodium carbonate buffer pH 10.2, 0.02ml of tissue homogenate and 0.03ml of epinephrine in 0.005 N HCl was used to initiate the reaction. The reference cuvette contained 2.95ml buffer, 0.03ml of substrate (epinephrine) and 0.02ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480nm for 5 minutes.

Determination of Catalase Activity

Serum catalase activity was determined according to the method of Beers and Sizer as described by Usuh et al. (2005) by measuring the decrease in absorbance at 240 nm due to the decomposition of H_2O_2 in a UV recording spectrophotometer. The reaction mixture (3 ml) contained 0.1ml of serum in phosphate buffer (50 mM, pH 7.0) and 2.9 ml of 30 mM H_2O_2 in phosphate buffer pH 7.0. An extinction coefficient for H_2O_2 at 240 nm of $40.0 M^{-1}cm^{-1}$ (Aebi, 1984) was used for the calculation. The specific activity of catalase was expressed as moles of H_2O_2 reduced per minute per mg protein.

Reduced Glutathione Determination

The reduced glutathione (GSH) content of organ tissue as non-protein sulphydryls was estimated according to the method described by Sedlak and Lindsay (1968). To the homogenate 10% TCA was added, centrifuged. 1.0ml of supernatant was treated with 0.5ml of Ellmans reagent (19.8mg of 5,5- dithiobisnitrobenzoic acid) (DTNB) in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412nm.

Lipid Peroxidation

Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of Buege and Aust (1978). 1.0ml of the supernatant was added to 2ml of (1:1:1) of TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24N HCl and 15% TCA) tricarboxylic acid thiobarbituric acid-hydrochloric acid reagent was boiled at $100^{\circ}C$ for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance read at 532nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA- complex of $1.56 \times 10^5 M^{-1} cm^{-1}$.

Statistical Analysis

Results are expressed as mean \pm SEM and differences between the groups were statistically determined by analysis of variance (ANOVA) followed by Tukey's test using graph pad prism 6.0. p -values < 0.05 were considered as statistically significant.

Results

Oral Acute Toxicity Study

There was mortality at all doses; 20%, 60%, 60%, 80% and 100% mortality at 1, 2, 3, 4 and 5 g/kg respectively. (Table 1)

Table 1: Probits and Percentage mortality in mice

| Extract Dose (mg/kg) | Mortality | % Mortality | Log Dose | Probit |
|----------------------|-----------|-------------|----------|--------|
| 1000 mg/kg | 1 | 20 | 3.0 | 4.1 |
| 2000 mg/kg | 3 | 60 | 3.30 | 5.25 |
| 3000 mg/kg | 3 | 60 | 3.40 | 5.25 |
| 4000 mg/kg | 4 | 80 | 3.60 | 5.84 |
| 5000 mg/kg | 5 | 100 | 3.69 | 7.33 |

Preliminary Phytochemical Screening

The preliminary phytochemical screening of the hydro-ethanolic root extract of *Z. zanthoxyloides* showed the presence of tannins, phlobatannins, saponins, cardiac glycosides, alkaloids, flavonoids, phenols and terpenoids.

Effect of Hydro-Ethanolic Root Extract of *Z. Zanthoxyloides* on Hematological Parameters

There was a significant ($p < 0.001$) reduction in the WBC count of rats treated with 2.5 mg/kg CP (1.0 ± 0.18), 75 mg/kg ZZ+CP (1.1 ± 0.23), 150 mg/kg ZZ+CP and 225 mg/kg ZZ+CP (1.5 ± 0.20) all compared to the control (7.2 ± 0.96). All other hematological indices were not significantly different from the control.

WBC count was significantly ($p < 0.05$) reduced in animals treated with 225 mg/kg ZZ only (3.80 ± 1.40) compared to the control (7.2 ± 0.96); a non-significant increase in WBC count in rats treated with 225 mg/kg ZZ only (3.80 ± 1.40) compared to 75 mg/kg ZZ+CP (1.1 ± 0.23), 150 mg/kg ZZ+CP (0.73 ± 0.33) and 225 mg/kg ZZ+CP (1.5 ± 0.20). (Table 2)

Table 2: Effect of the hydro-ethanolic root extract of *Z. zanthoxyloides* on hematological parameters

| | WBC $\times 10^9/L$ | MCV (g/L) | MCH (pg) | MCHC (g/dL) | HGB (g/dL) | RBC $\times 10^{12}/L$ | PCV (%) | PLT ($10^5/\mu L$) |
|--------------------|------------------------|-----------------|-----------------|-----------------|-----------------|---------------------------|-----------------|----------------------|
| CONTROL | 7.2 ± 0.96 | 61.0 ± 1.90 | 20.0 ± 0.81 | 32.0 ± 0.30 | 11.0 ± 1.40 | 5.60 ± 0.50 | 34.0 ± 4.00 | 656.0 ± 13.70 |
| 2.5 mg/kg CP | 1.0 ± 0.18^c | 56.0 ± 2.10 | 19.0 ± 0.60 | 34.0 ± 0.23 | 11.0 ± 0.81 | 5.60 ± 0.42 | 31.0 ± 2.40 | 505 ± 132.00 |
| 75 mg/kg ZZ+CP | 1.1 ± 0.23^c | 59.0 ± 2.0 | 19.0 ± 0.42 | 33.0 ± 1.20 | 9.0 ± 0.73 | 4.70 ± 0.36 | 28.0 ± 1.30 | 501.0 ± 151.00 |
| 150 mg/kg ZZ+CP | 0.73 ± 0.33^c | 57.0 ± 1.10 | 19.0 ± 0.35 | 33.0 ± 0.71 | 11.0 ± 0.35 | 5.90 ± 0.13 | 34.0 ± 0.38 | 503.0 ± 172.00 |
| 225 mg/kg | 3.8 ± 1.40^f | 58.0 ± 1.70 | 19.0 ± 0.70 | 33.0 ± 0.31 | 11.0 ± 0.54 | 5.40 ± 0.19 | 34.0 ± 1.90 | 723.0 ± 11.60 |
| 225 mg/kg ZZ+CP | 1.5 ± 0.20^d | 57.0 ± 1.50 | 20.0 ± 0.33 | 35.0 ± 0.76 | 11.0 ± 0.20 | 6.0 ± 0.49 | 31.0 ± 0.95 | 604.0 ± 108.00 |

Values are expressed as mean \pm SEM (n=5). * $p < 0.05$, **** $p < 0.0001$ versus control (One Way ANOVA followed by Tukey's *posthoc* multiple comparison test).

Effect of the hydro-ethanolic root extract of *Z. zanthoxyloides* on biochemical parameters I

There was no statistically significant difference in the levels of all parameters assessed compared to the control (Table 3A).

Table 3A: Effect of the hydro-ethanolic root extract of *Z. zanthoxyloides* on biochemical parameters

| | AST, IU | ALT, IU | ALP, IU | BIL-T, mg/dL | CREA, mg/dL | UREA, mg/dL | ALB, g/L | TP, g/dL |
|--------------------|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|------------------|
| CONTROL | 29.33 ± 2.91 | 6.00 ± 3.06 | 1.00 ± 1.15 | 0.53 ± 0.12 | 1.10 ± 0.06 | 3.67 ± 1.67 | 2.30 ± 0.17 | 9.33 ± 14.83 |
| 2.5 mg/kg CP | 5.33 ± 2.03 | 3.00 ± 3.51 | 1.67 ± 5.46 | 0.53 ± 0.07 | 1.13 ± 0.07 | 0.00 ± 4.51 | 3.17 ± 0.16 | 5.73 ± 0.48 |
| 75 mg/kg ZZ+CP | 43.67 ± 2.73 | 2.00 ± 5.03 | 5.67 ± 8.84 | 0.57 ± 0.09 | 1.17 ± 0.15 | 7.33 ± 4.26 | 3.03 ± 0.47 | 5.13 ± 0.58 |
| 150 mg/kg ZZ+CP | 5.33 ± 4.10 | 1.00 ± 5.03 | 0.00 ± 5.57 | 0.47 ± 0.07 | 0.83 ± 0.03 | 4.00 ± 2.08 | 3.23 ± 0.27 | 5.27 ± 0.58 |
| 225 mg/kg ZZ | 5.67 ± 3.57 | 2.67 ± 3.18 | 7.00 ± 4.58 | 0.47 ± 0.03 | 0.97 ± 0.03 | 1.33 ± 6.39 | 3.17 ± 0.33 | 4.67 ± 0.26 |
| 225 mg/kg ZZ+CP | 2.67 ± 8.45 | 0.00 ± 6.66 | 4.00 ± 6.66 | 0.57 ± 0.15 | 1.00 ± 0.06 | 5.33 ± 5.78 | 2.97 ± 0.09 | 5.23 ± 0.19 |

Values are expressed as mean \pm SEM (n=5). No significant difference from the control

Effect of the hydro-ethanolic root extract of *Z. zanthoxyloides* on biochemical parameters II

LDLc was significantly ($p < 0.0001$, 0.001) reduced at 75 mg/kg ZZ+CP (91.00 ± 6.11), 225 mg/kg ZZ (95.34 ± 4.19) and 225 mg/kg ZZ+CP (92.00 ± 5.13) compared to the control (128.67 ± 2.60). There was significant ($p < 0.05$) reduction at 225 mg/kg ZZ (95.34 ± 4.19), significant ($p < 0.01$) reduction at 225 mg/kg ZZ+CP (92.00 ± 5.13) compared to 150 mg/kg ZZ+CP (117.00 ± 9.17). CHOL was significantly ($p < 0.0001$) reduced at 75 mg/kg ZZ+CP (128.0 ± 7.55), 225 mg/kg ZZ (134.33 ± 12.44) and 225 mg/kg ZZ+CP (130.33 ± 6.70) compared to the control (177.33 ± 6.77). A nonsignificant reduction in triglycerides was seen at all doses used compared to CP administered and control rats. (Table 3B)

Effect of hydro-ethanolic root extract of *Z. zanthoxyloides* on anti-oxidant enzymes

SOD showed a significant ($p < 0.001$) increase at 75 mg/kg ZZ+CP (2.90 ± 0.21) when compared with the CP treated (1.20 ± 0.32) rats, a significant ($p < 0.001$) reduction at 150 mg/kg ZZ+CP (1.40 ± 0.11), 225 mg/kg ZZ only (0.93 ± 0.11) compared to rats treated with 75 mg/kg ZZ+CP (2.90 ± 0.21). CAT levels at 150 mg/kg ZZ+CP (19.0 ± 2.20) were significantly ($p < 0.05$) increased compared to 75 mg/kg ZZ+CP (12.0 ± 0.78). A significant ($p < 0.05$) increase was observed at 150 mg/kg ZZ+CP (19.0 ± 2.20) compared to 75 mg/kg ZZ+CP (12.0 ± 0.78), 225 mg/kg+CP (12.0 ± 0.46). GPx levels was significantly ($p < 0.001$) increased in rats treated with 75 mg/kg ZZ+CP (0.89 ± 0.058) compared to the control (0.49 ± 0.046), and at $p < 0.01$ for rats treated with 75mg/kg ZZ+CP (0.89 ± 0.058)

compared to CP treated (0.36±0.095). There was significant ($p < 0.01$) reduction at 150 mg/kg+CP (0.41±0.034), $p < 0.001$ at 225 mg/kg+CP (0.39±0.055) compared to 75 mg/kg+CP (0.89±0.058).

The levels of MDA in rats treated with 225 mg/kg ZZ only (0.054±0.0026) was significantly ($p < 0.01$) reduced compared to 150 mg/kg+CP (0.12±0.013). (Table 4)

Table 3B: Effect of the hydro-ethanolic extract of *Z. zanthoxyloides* on biochemical parameters II

| | HDLc, mg/dL | LDLc, mg/dL | CHOL, mg/dL | TG, mg/dL |
|-----------------|--------------|------------------------------|--------------------------------|--------------|
| CONTROL | 39.33 ± 4.41 | 128.67 ± 2.60 | 177.33 ± 6.77 | 48.67 ± 7.80 |
| 2.5 mg/kg CP | 34.33 ± 2.85 | 93.33 ± 4.67 ^c | 136.67 ± 8.01 ^c | 44.33 ± 4.10 |
| 75 mg/kg ZZ+CP | 28.67 ± 1.33 | 91.00 ± 6.11 ^c | 128.00 ± 7.55 ^c | 40.67 ± 5.90 |
| 150 mg/kg ZZ+CP | 26.67 ± 2.40 | 117.00 ± 9.17 ^b | 151.67 ± 11.22 ^{c, a} | 39.33 ± 0.88 |
| 225 mg/kg ZZ | 31.33 ± 3.67 | 95.34 ± 4.19 ^{d, a} | 134.33 ± 12.44 ^c | 38.00 ± 1.16 |
| 225 mg/kg ZZ+CP | 31.00 ± 2.00 | 92.00 ± 5.13 ^{c, β} | 130.33 ± 6.70 ^{c, a} | 37.33 ± 3.71 |

Values are expressed as Mean ± SEM (n=5). *** $p < 0.001$, **** $p < 0.0001$ versus control; ^a $p < 0.05$, ^b $p < 0.01$ vs. 75 mg/kg ZZ + 2.5 mg/kg CP; ^a $p < 0.05$, ^β $p < 0.01$ versus 150 mg/kg ZZ + 2.5 mg/kg CP. (Two-way ANOVA followed by Tukey's *posthoc* multiple comparison test).

Table 4: Effect of the hydro-ethanolic root extract of *Z. zanthoxyloides* on the level of antioxidant enzymes

| | GSH (U/mg protein) | SOD (U/mg protein) | CAT (U/mg protein) | MDA (U/mg protein) | GPx (U/mg protein) |
|-----------------|--------------------|---------------------------|---------------------------|------------------------|---------------------------|
| CONTROL | 0.23±0.09 | 1.60±0.15 | 11.0±1.40 | 0.02±0.00 | 0.49±0.05 |
| 2.5 mg/kg CP | 0.06±0.02 | 1.20±0.32 | 16.0±1.80 | 0.02±0.00 | 0.36±0.09 ^d |
| 75 mg/kg ZZ+CP | 0.21±0.05 | 2.90±0.21 ^{c, a} | 12.0±0.78 ^{1, a} | 0.04±0.02 | 0.89±0.06 ^{c, a} |
| 150 mg/kg ZZ+CP | 0.05±0.01 | 1.40±0.11 ^d | 19.0±2.20 ¹ | 0.12±0.01 ^c | 0.41±0.03 ^c |
| 225 mg/kg ZZ | 0.19±0.04 | 0.93±0.11 ^d | 13.0±0.058 | 0.05±0.00 | 0.28±0.03 ^c |
| 225 mg/kg ZZ+CP | 0.11±0.06 | 1.30±0.18 | 12.0±0.46 ^{1, a} | 0.02±0.00 ^e | 0.39±0.05 ^d |

Values are expressed as Mean ± SEM (n=5) * $p < 0.05$ versus control, ^a $p < 0.05$ vs 150 mg/kg+CP, ¹ $p < 0.01$ versus CP and control, ** $p < 0.01$ versus 150 mg/kg+CP, 75 mg/kg+CP, *** $p < 0.001$ versus 75 mg/kg+CP, **** $p < 0.0001$ vs control, 225mg/kg (Two Way ANOVA followed by Tukey's *posthoc* multiple comparison test)

Discussion

This study was carried out to evaluate the preventive or positive modulatory roles of hydroethanolic root extract of *Z. zanthoxyloides* against cyclophosphamide induced myelosuppression and oxidative stress.

In the acute toxicity study, oral administration of the extract of *Z. zanthoxyloides* produced mortality at all doses. The LD₅₀ of the extract was found to be 1698.2 mg/kg. As documented by the Hodge and Sterner scale of toxicity, this value showed that the extract of *Z. zanthoxyloides* is slightly toxic to rats.

The phytochemical analysis of the plant extract showed the presence of tannins, saponins, alkaloids and flavonoids, glycosides, terpenoids and phenols. The antioxidant and antilipoperoxidative properties of this extract could be attributed to its constituent flavonoids and other polyphenolics as these phytochemicals have been widely reported to possess antioxidant and antilipoperoxidative activities (Roy et al., 2005; Yang et al., 2010).

Hematological analysis following sub-acute oral administration of the hydro-ethanolic extract of *Z. zanthoxyloides* showed that there was significant reduction in the WBC count of rats treated with the extract and cyclophosphamide. WBC's which are produced in the bone marrow are an important part of the immune system and the body's natural weapons to fight off infections. A low WBC count is often linked to problems with the bone marrow and the inability to make enough white blood cells. This suggests that the extract is unable to improve immune suppression in treated rats. Extract of *Z. zanthoxyloides* may play no positive modulatory role in cyclophosphamide induced low white blood cell counts probably due to the ability of cyclophosphamide to elicit its toxic effects by acting directly on rapidly dividing cells like white blood cells and direct DNA damage, hence the inability of the extract to play any beneficial roles. Ogunbolude et al. (2014) reported that *Z. zanthoxyloides* showed cytotoxic and genotoxic effects *in vitro* on human leukocyte at a concentration of 150µg/ml, hence suggesting the possibility of a combined toxic role of the extract and cyclophosphamide on white blood cells.

The imbalance between antioxidant and pro-oxidant system is responsible for oxidative stress. The antioxidant system consists of enzymatic components comprising CAT, SOD, and non-enzymatic components containing GSH, vitamins A, C, E and other exogenous sources (Adekunle et al., 2012). Findings from this study showed a significant increase in CAT, SOD and GPx respectively. Majority of diseases are mainly linked to oxidative stress due to free radicals (Gutteridge, 1995). Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism (Tiwari, 2001). The most common reactive oxygen

species (ROS) include superoxide (O_2^-) anion, hydrogen peroxide (H_2O_2), peroxy (ROO^\cdot) radicals, and reactive hydroxyl (OH^\cdot) radicals. The nitrogen derived free radicals are nitric oxide (NO^\cdot) and peroxynitrite anion ($ONOO^-$) (Lobo et al., 2010). ROS have been implicated in over a hundred of disease states which range from arthritis and connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome (Joyce, 1987). In treatment of these diseases, antioxidant therapy has gained an immense importance. Antioxidants have been reported to prevent oxidative damage by free radical and ROS, and may prevent the occurrence of disease, cancer and aging. It can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers (Buyukokuroglu et al., 2001; Shahidi and Wanasundara, 1992). The antioxidant potentials of the extract suggests its probable usefulness in exerting multiple biological effects including free radical scavenging abilities, anti-inflammatory and anti-carcinogenic effects possibly due to the presence of flavonoids and phenolic compounds (Miller, 1996). The selenium-containing enzyme GPx protects cells against ROS. SOD and catalase are present in all oxygen-metabolizing cells and their function is to provide a defense against the potentially damaging reactivities of superoxide and hydrogen peroxide.

Several studies indicates that cyclophosphamide has a pro-oxidant character and generation of oxidative stress after administration leads to decrease in the activities of antioxidant enzymes and increase in lipid peroxidation in liver, lung and serum of mice and rats (Perez et al., 1991; Barja de Quiroga et al., 1990; Patel and Block, 1985). The increased level of antioxidant enzymes demonstrates the oxidant scavenging potentials especially at low and moderate doses of the extract and thus could be beneficial in conditions requiring cyclophosphamide therapy.

On assessment of serum biochemical markers, LDL and CHOL were significantly decreased compared to vehicle treated control. This is in agreement with findings by Oyewole et al. (2012) who found out that fresh leaves and stem bark extracts of *Z. zanthoxyloides* caused significant reduction in the concentrations of serum total cholesterol and LDL-cholesterol in rats administered. Cholesterol is essential for proper functioning of the cell membrane as well as being very essential for vitamin D and steroid hormone synthesis. It is transported by LDL and HDL and significant lowering of total cholesterol and rise in HDL-C is a very desirable biochemical state for prevention of atherosclerosis and ischaemic conditions (Schwenke and Carew, 1989; Luc and Fruchart, 1991). The significant reduction in LDL and CHOL could be ascribed to the presence of saponin which finds usage in conditions like hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss (Mandal et al., 2005; Manjunatha, 2006). Saponins are known anti-nutritional factors, which lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase its fecal excretion (Nimenibo-uadia, 2003; Jamea et al., 2010; Rotimi et al., 2011). Increased bile acid excretion is offset by enhanced bile acid synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol (James et al., 2010). This suggests that the extract could be beneficial in hypercholesterolemia and cardiovascular conditions triggered by abnormal cholesterol metabolism, an effect which may be mediated through improvement of antioxidant indices. The link between free radicals and disease processes has led to considerable research to develop nontoxic drugs that can scavenge free radicals hence the antioxidant potentials of the extract may confer on it the potential of being a very useful candidate in malignant conditions requiring cyclophosphamide therapy and with comorbid abnormal cholesterol metabolism.

Conclusion

The result obtained in this study suggests that the hydro-ethanolic root extract of *Z. zanthoxyloides* may have positive modulatory roles against cyclophosphamide induced toxic effects probably due to induction of the antioxidant system. However, it was unable to combat and/or improve cyclophosphamide induced myelosuppression.

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Conflict of Interests: The authors declare that there are no conflicts of interest in respect of this study.

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